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Comparison of Antimicrobial Susceptibility Profiles of Escherichia Coli Strains Isolated from Broiler Chickens and Patients with Acute Intestinal Infections

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Abstract Amid the growing global threat of antibiotic resistance, the spread of resistant Escherichia coli strains in both clinical and veterinary settings is a matter of particular concern. In this study, we conducted a comparative analysis of E. coli strains isolated from patients with acute intestinal infections and from broiler chickens. The research was carried out at the Antimicrobial Resistance Center laboratory of the Republican Specialized Scientific-Practical Medical Center for Epidemiology, Microbiology, and Infectious and Parasitic Diseases of the Republic of Uzbekistan. Special attention was given to determining antimicrobial resistance profiles using the disk diffusion method, as well as to identifying resistance genes associated with aminoglycoside and β-lactam antibiotics through molecular techniques. Using polymerase chain reaction (PCR), we detected and characterized β-lactamase genes (bla_TEM, bla_SHV, bla_CTX-M, bla_VIM, bla_NDM, bla_KPC) and aminoglycoside-modifying enzyme genes (aac(6')-II, aph(3')-VI). The bla_CTX-M gene was detected at a high frequency (60.7%) among strains isolated from patients with acute intestinal infections, whereas bla_TEM was the most prevalent in broiler chicken isolates (89.3%). A strong correlation was observed between genotypic and phenotypic resistance profiles, underscoring the critical role of molecular surveillance in clinical microbiology. The obtained data reveal a high prevalence of multidrug resistance and confirm the circulation of shared genetic determinants among Escherichia coli strains isolated from both patients with acute intestinal infections and broiler chickens. This underscores the need for a comprehensive, integrated approach to antimicrobial resistance surveillance within the framework of the One Health concept.

Keywords Escherichia coli, Strains, Acute intestinal infection, Antimicrobial resistance

1. Introduction

Antimicrobial resistance (AMR) is now recognized as one of the most serious threats to global public health. Gram-negative bacteria - particularly Escherichia coli - are of special concern due to their remarkable ability to rapidly adapt to antimicrobial therapy through the acquisition and horizontal transfer of resistance genes. Of particular clinical significance are E. coli strains that produce extended-spectrum β-lactamases (ESBLs) and carbapenemases. These enzymes confer resistance to key classes of β-lactam antibiotics, including extended-spectrum cephalosporins and carbapenems - often considered last-resort treatments for severe infections. Such resistance mechanisms severely complicate the management of infections in both human and veterinary medicine and facilitate the spread of resistant pathogens through food chains, the environment, and direct contact [1].

The study of Escherichia coli strains isolated from humans with acute intestinal infections (AII) and from livestock—particularly broiler chickens—has become increasingly relevant, as these reservoirs are likely interconnected within the framework of the One Health concept. Antibiotic resistance genes encode proteins that enable bacterial survival in the presence of antimicrobial agents. The primary resistance mechanisms include: Antibiotic hydrolysis (e.g., β-lactamases such as bla_TEM, bla_SHV, bla_CTX-M, bla_KPC); Target modification (e.g., altering antibiotic binding to the 30S ribosomal subunit, aac(6')-II); Active efflux of the

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drug (efflux pumps); Changes in cell membrane permeability; Antibiotic modification (e.g., acetylation, phosphorylation — aph(3')-VI, adenylation).

Resistance genes may be located both on the bacterial chromosome and on mobile genetic elements, such as plasmids (e.g., bla_TEM, bla_NDM, bla_CTX-M). Gene transfer can occur: Horizontally — between different bacteria (via conjugation, transformation, or transduction); Vertically — from parent cell to progeny during cell division.

The mobility of these resistance genes renders the antimicrobial resistance problem particularly acute, as pathogens can rapidly acquire resistance even in the absence of prolonged antibiotic exposure.

Molecular detection of resistance genes such as bla_TEM, bla_SHV, bla_CTX-M, bla_VIM, bla_NDM, bla_KPC, aac(6')-II, and aph(3')-VI represents an important tool for monitoring the spread of resistance and assessing the risk of transmission across different populations.

2. Materials and Methods

To achieve the stated objectives, the research was conducted in the laboratory of the **Center for Antimicrobial Resistance (CAMR)** at the RSSPMCEMIPD within the framework of the state grant PZ: 20170928351 “Development of a system for predicting and preventing adverse effects of alimentary factors on human health based on the determination of resistance phenotypes and common patterns of microbial susceptibility to antimicrobial agents in patients with diarrhea and in farm animals” (January 3, 2018 – December 31, 2020).

The study of morphological, tinctorial, and biochemical properties of E. coli strains was carried out in accordance with WHO protocols [2]. Biochemical activity was determined by inoculation of cultures into semi-liquid media containing various carbohydrates, alcohols, and amino acids: glucose, mannitol, dulcitol, urea, arabinose, xylose, citrate, acetate, malonate, phenylalanine, and lysine. Indole formation was determined in broth using the Morel reagent method [3]. Antimicrobial susceptibility testing was performed by the disk diffusion method, and the interpretation of results was carried out according to the EUCAST recommendations (2021, version 11.0) [4]. Mueller–Hinton agar (GEETA PHARMA, India) and antibiotic discs manufactured by Liofilchem S.r.l. (Italy) were used throughout the testing. The susceptibility of E. coli was tested against the following antimicrobial agents: β-lactams (ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, ceftriaxone, imipenem, meropenem, ertapenem, doripenem), and aminoglycosides (amikacin). For internal quality control of the experiments, the reference strain E. coli ATCC 25922 was used.

The resistance mechanisms of E. coli strains to β-lactam and aminoglycoside antibiotics were studied using molecular genetic methods: PCR with electrophoretic detection. Genes encoding β-lactamases of broad and extended spectrum families (bla_SHV, bla_TEM, bla_CTX-M), carbapenemases (bla_KPC) and metallo-β-lactamases (bla_NDM, bla_VIM), as well as aminoglycoside resistance genes (aph(3')-VI, aac(6')-II) were detected.

DNA extraction. Bacterial DNA was isolated using the thermal lysis method with sterile water. Five to six colonies from a 24-hour culture of the test microorganism, grown on solid agar medium, were transferred into a 1.5 mL microcentrifuge tube containing 1,000 μL of nutrient broth. The suspension was vortexed briefly to disperse the cells and then centrifuged (Microspin 12, Biosan) at 13,000 × g for 5 minutes to pellet the bacteria. The supernatant was discarded, and the pellet was resuspended in 400 μL of sterile water and vortexed (MX-S, DLAB). Tubes were incubated in a dry block thermostat (TDB-120, Biosan) for 10 min at 95°C, followed by centrifugation at 13,000 g for 5 min. The supernatant containing released DNA was transferred to a clean tube. DNA quality and concentration were measured using a BioSpec-nano spectrophotometer.

PCR analysis of E. coli resistance genes (bla_SHV, bla_TEM, bla_CTX-M, bla_KPC, bla_VIM, bla_NDM, aph(3')-VI, aac(6')-II), was performed using lyophilized GenePak™ PCR Core reagents (LLC “Izogen Laboratory,” Russia). Each reaction tube contained lyophilized Taq DNA polymerase and dNTPs. The final reaction volume was 20 μL, including the added template DNA.

DNA from the reference strain Escherichia coli ATCC 25922 was used as a negative control. Amplification reactions were performed using a Veriti™ Dx 96-Well Thermal Cycler (Applied Biosystems). Gene-specific primers targeting the following resistance determinants were employed: bla_SHV, bla_TEM, bla_CTX-M, bla_KPC, bla_VIM, bla_NDM, aph(3')-VI and aac(6')-II (primer sequences and reaction conditions are provided in Table 1).

The PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide, and the results were interpreted based on the presence and size of the expected DNA bands.

3. Results and Discussion

A total of 28 Escherichia coli strains were isolated from the intestinal contents of broiler carcasses collected during slaughter at Farm No. 1 in Tashkent. Using random sampling, 10 carcasses from apparently healthy birds (mean body weight: 1,000 ± 100 g) were selected, and each was processed for bacteriological analysis on the day of collection. Additionally, 28 E. coli strains were isolated from stool samples of patients hospitalized with acute intestinal infections (AII) at the Republican Specialized Scientific-Practical Medical Center for Epidemiology, Microbiology, and Infectious and Parasitic Diseases (RSSPMCEMIPD).

Table 1. Primers used in amplification reactions

	Gene	Primer name	Nucleotide sequence (5′–3′)	b.p.	Autors
1	blaSHV	SHV-F1	GGCCGCGTAGGCATGATAGA	714	AL-Mahfood h 2021
		SHV-R1	CCCGGCGATTTGCTGATTTC		
2		SHV-F2	CTTTATCGGCCCTCACTCAA	237	Rasha Hamed El sherif 2012
		SHV-R2	AGGTGCTCATCATGGGAAAG		
3	blaTEM	TEM-F1	CAGCGGTAAGATCCTTGAGA	643	AL-Mahfood h 2021
		TEM-R1	ACTCCCCGTCGTGTAGATAA		
4		TEM-F2	CGCCGCATACACTATTCTCAGAATGA	445	Tsobeng, O. D 2024
		TEM-R2	ACGCTCACCGGCTCCAGATTTAT		
5	blaCTX-M	CTX-M_F	ATGTGCAGYACCAGTAARGTKATGGC	593	Tsobeng, O. D. 2024
		CTX-M_R	TGGGTRAARTARGTSACCAGAAYCAGCGG		
6	blaKPC	KPC-F1	CATTCAAGGGCTTTCTTGCTGC	498	Mahmoud, N. E 2020
		KPC-R1	ACGACGGCATAGTCATTTGC		
7		KPC-F2	ATGTCACTGTATCGCCGTCT	498	Bagaya, J 2023
		KPC-R2	TTTTCAGAGCCTTACTGCCC		
8	blaVIM	VIM-F1	GGTGTTTGGTCGCATATCGCAA	502	Mahmoud, N. E 2020
		VIM-R1	-ATTCAGCCAGATCGGCATCGGC		
9		VIM-F2	GATGGTGTTTGGTCGCAT	390	Bagaya, J 2023
		VIM-R2	CGAATGCGCAGCACCAG		
10	blaNDM	NDM-F	GGTTTGGCGATCTGGTTTTC	621	Bagaya, J 2023
		NDM-R	CGGAATGGCTCATCACGATC		
11	aph(3')-VI	Aph-F	ATGGAATTGCCCAATATTATT	780	Hu, X 2013
		Aph-R	TCAATTCAATTCATCAAGTTT		
12	aac(6')-II	Aac-F	CGACCATTTCATGTCC	542	Hu, X 2013
		Aac-R	GAAGGCTTGTCGTGTTT		

Note: bp – base pairs, Y – C or T, R – A or G, K – G or T, S – G or C.

Table 2. Antimicrobial susceptibility of E. coli strains isolated from broiler chickens and AII patients

	E. coli from broiler chickens				E.coli from patients AII			
Antibiotics	**Quantity**	R (n) %	I (n) %	RI(n) %	**Quantity**	R (n) %	I (n) %	RI(n) %
Penicillins								
AMP_ED10	28	27 (96,4)	0 (0,0)	27 (96,4)	28	24 (85,7)	0 (0,0)	24 (85,7)
TZP_ED36	28	4 (14,2)	1 (3,6)	5 (17,8)	28	8 (28,6)	5 (17,8)	13 (46,4)
Beta-lactam/inhibitor								
AMC_ED20	28	27 (96,4)	0 (0,0)	27 (96,4)	28	24 (85,7)	0 (0,0)	24 (85,7)
Cephems								
CTX_ED5	28	0 (0,0)	0 (0,0)	0 (0,0)	28	20 (71,4)	0 (0,0)	20 (71,4)
CAZ_ED10	28	15 (53,6)	1 (3,6)	16 (57,2)	28	27 (96,4)	0 (0,0)	27 (96,4)
FEP_ED30	28	0 (0,0)	1 (3,6)	1 (3,6)	28	20 (71,4)	0 (0,0)	20 (71,4)
CRO ED30	28	0 (0,0)	0 (0,0)	0 (0,0)	28	21 (75,0)	0 (0,0)	21 (75,0)
Penems								
IPM_ED10	28	3 (10,8)	0 (0,0)	3 (10,8)	28	0 (0,0)	18 (64,3)	18 (64,3)
MEM_ED10	28	0 (0,0)	0 (0,0)	0 (0,0)	28	0 (0,0)	0 (0,0)	0 (0,0)
ETP_ED10	28	0 (0,0)	0 (0,0)	0 (0,0)	28	2 (7,1)	0 (0,0)	2 (7,1)
DOR_ED10	28	13 (46,4)	0 (0,0)	13 (46,4)	28	14 (50,0)	10 (35,7)	24 (85,7)
Aminoglycosides								
AMK_ED30	28	0 (0,0)	0 (0,0)	0 (0,0)	28	2 (7,2)	0 (0,0)	2 (7,2)

Note: AMP_ED10 – ampicillin, AMC_ED20 – amoxicillin/clavulanic acid, TZP_ED36 – piperacillin/tazobactam, CTX_ED5 – cefotaxime, CAZ_ED10 – ceftazidime, FEP_ED30 – cefepime, CRO_ED30 – ceftriaxone, IPM_ED10 – imipenem, MEM_ED10 – meropenem, ETP_ED10 – ertapenem, DOR_ED10 – doripenem, AMK_ED30 – amikacin

To assess possible differences in the frequency of resistance between E. coli strains isolated from broilers and those from AII patients, strains with moderate and high resistance levels were combined into a single category, "non-susceptible." Poultary isolates (Table 2) showed higher resistance to ampicillin and amoxicillin/clavulanic acid — 96.4% each, compared with human isolates (85.7% and 93.1%, respectively). In contrast, human-derived strains exhibited significantly higher resistance to third- and fourth-generation cephalosporins: cefotaxime (71.4%), ceftriaxone (75.0%), cefepime (71.4%), and ceftazidime (96.4%). Notably, cephalosporin resistance was largely absent in poultry isolates, with the exception of ceftazidime (57.2%). Differences were also noted in resistance to carbapenems. Marked differences were also observed for carbapenems. Among human isolates, resistance to imipenem and doripenem was 64.3% and 85.7%, respectively, whereas in broiler isolates, these values were considerably lower (10.8% and 46.4%, respectively).

Both sources were characterized by preserved susceptibility to meropenem and ertapenem (with complete susceptibility among human isolates and absence of resistance among poultry isolates).

The observed differences in the antibiotic resistance profiles of E. coli strains isolated from broiler chickens and from patients with acute intestinal infections are most likely explained by variations in the schemes and intensity of antimicrobial use in veterinary and clinical practice. The high resistance of broiler chicken isolates to ampicillin and amoxicillin/clavulanic acid can presumably be attributed to the widespread use of these antibiotics in poultry farming for the prevention and treatment of bacterial infections. In contrast, the higher resistance rates to third- and fourth-generation cephalosporins and carbapenems observed in patient isolates may reflect the predominant use of these antibiotics in hospitals for the treatment of severe infections, creating selective pressure that facilitates the spread of corresponding resistance mechanisms.

Furthermore, the identified differences may be associated with the circulation and accumulation of resistance genes in microbial populations, variations in poultry farming conditions and clinical patient flows, as well as differences in antibiotic usage monitoring and regulation systems in veterinary and healthcare settings.

In Jamaica, poultry E. coli exhibited resistance to β-lactams, ampicillin, and amoxicillin/clavulanic acid at rates of 20.6% and 2.9%, respectively [5]. In a study of 370 E. coli strains collected from clinical samples in China, high resistance to cefotaxime (46.22%) was reported [6]. Another study in southeastern Austria analyzed over 120,000 E. coli strains isolated from hospitalized patients, showing that the proportion of resistant isolates increased over time, particularly for ampicillin (25.4% in 1998 to 40% in 2013), cefotaxime (0.1%–6.7%), ceftazidime (0.3%–14.2%), and ciprofloxacin (4.3%–16.7%) [7].

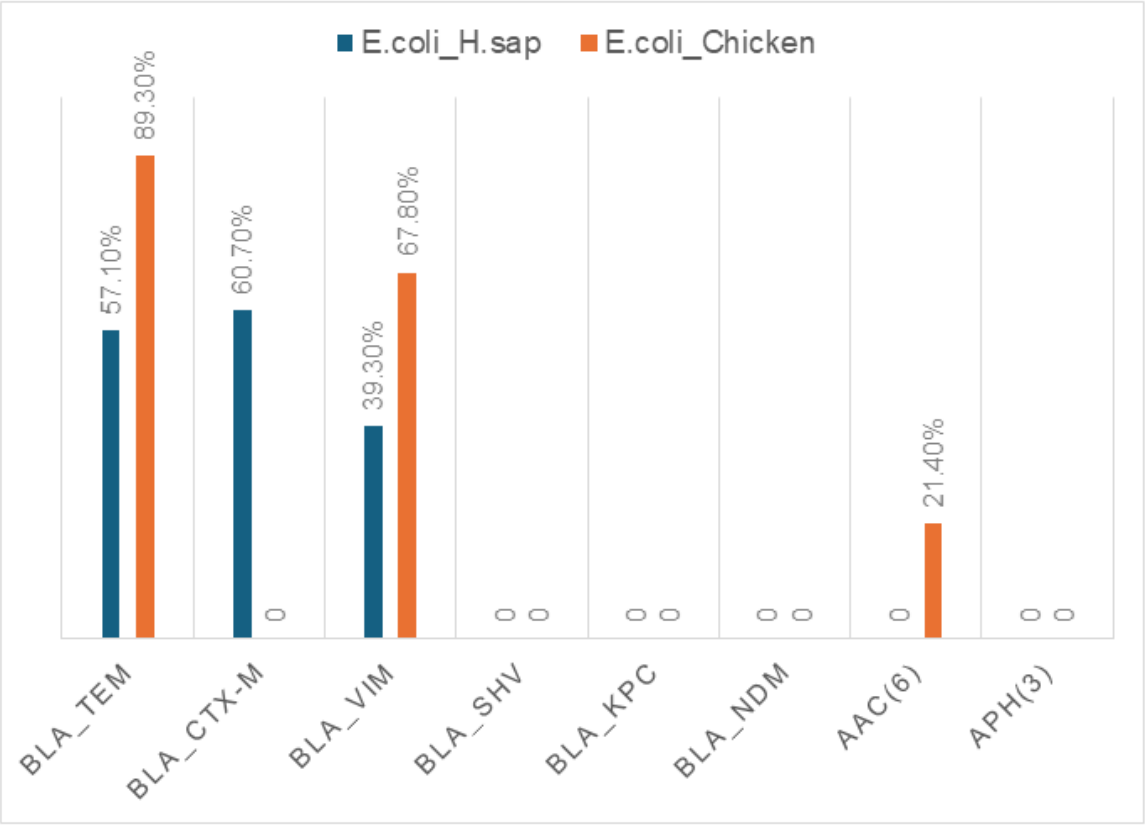

Figure 1. Comparative chart of resistance genes in AII patients and broiler chickens

Thus, third- and fourth-generation cephalosporins and carbapenems are commonly used for hospital treatment, which explains the high resistance to these classes of antibiotics. In poultry farming, however, ampicillin, tetracyclines, and fluoroquinolones are more frequently applied, indicating resistance to these classes.

Extended-spectrum β-lactamases (ESBLs) produced by Enterobacteriaceae represent a widespread source of antimicrobial resistance in both animals and humans [8]. E. coli isolates producing ESBLs contribute to life-threatening infections worldwide. The global prevalence of such E. coli was reported to be 33.0% in humans and 33.5% in animals. Moreover, three main ESBL resistance genes — bla_SHV, bla_CTX-M, and bla_TEM — have been identified in both humans and animals [9].

Among E. coli strains isolated from patients with acute intestinal infections (AII) and from broiler chickens, the prevalence of the genes bla_TEM, bla_CTX-M, bla_SHV, bla_VIM, bla_NDM, bla_KPC, aac(6′)-II, and aph(3′)-VI was analyzed by PCR.

The bla_TEM gene was detected in 89.3% of poultry isolates, compared to 57.1% of strains from AII patients, a difference of 32.2%. The prevalence of the bla_SHV gene was nearly identical in both groups, at 3.6%. The bla_CTX-M gene, associated with resistance to third- and fourth-generation cephalosporins (cefotaxime, ceftriaxone), was not detected in poultry isolates, whereas it was present in 60.7% of isolates from AII patients (Fig. 1). This likely reflects the broader use of cephalosporins in clinical practice among the population [10].

The bla_NDM and bla_KPC genes, responsible for carbapenem resistance, were not amplified in either of the studied groups, which most likely indicates their absence in these strains. In contrast, the bla_VIM gene was detected 25% more frequently in isolates obtained from chickens compared to those from patients.

When assessing the prevalence of aminoglycoside resistance gene aac(6′)-II and aph(3′)-VI genes, the aac(6′)-II gene was detected in 21.4% of Escherichia coli strains isolated from broiler chickens, whereas neither gene was detected in strains from patients with acute intestinal infections (AII).

E. coli strains obtained from AII patients demonstrated a high level of resistance to cephalosporins (ceftriaxone, cefotaxime, cefepime) and carbapenems (imipenem, doripenem). Such resistance is likely due to the widespread use of these drugs in clinical practice[11]. In contrast, the bla_CTX-M gene was not identified in broiler chicken isolates, which likely reflects the limited use of third- and fourth-generation cephalosporins in veterinary practice.

The high prevalence of bla_TEM and aac(6′)-II genes in poultry indicates the presence of strains resistant to β-lactam antibiotics and aminoglycosides in this population. A likely reason for this is the prophylactic use of antibiotics in poultry farms [12].

According to the results of microbiological and molecular-genetic studies (Figs. 2, 3), a comparative analysis of resistance to three main groups of antibiotics—β-lactams, carbapenems, and aminoglycosides—was conducted. No significant differences between the two methods were observed regarding resistance to β-lactam antibiotics.

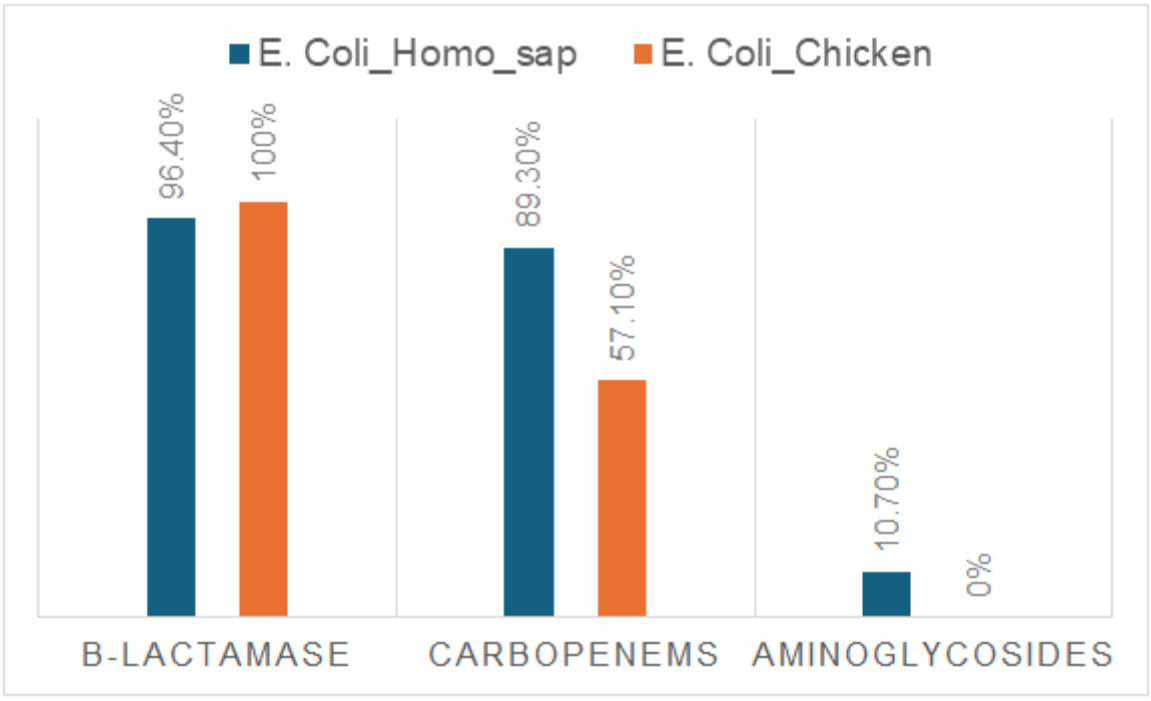

Figure 2. Microbiological analysis of resistance of E. coli strains to three groups of antimicrobial agents

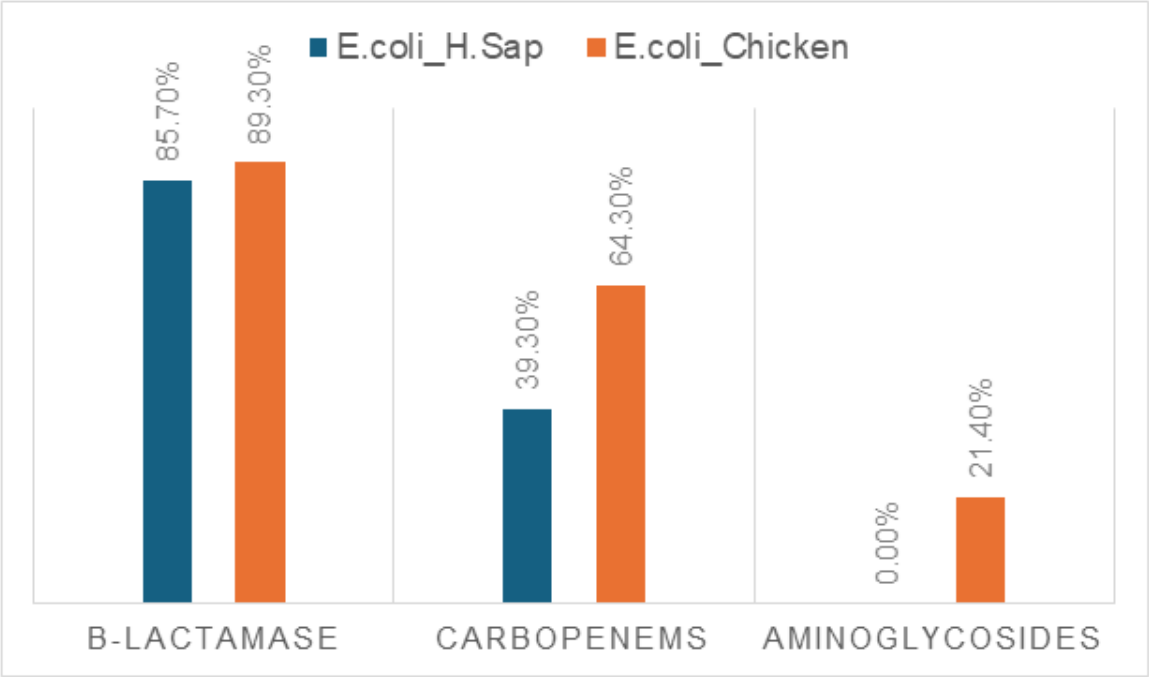

Figure 3. Molecular genetic analysis of resistance of E. coli strains to three groups of antimicrobial agents

When assessing carbapenem resistance, a marked discrepancy was observed between phenotypic and genotypic results. Among Escherichia coli strains isolated from patients with acute intestinal infections (AII), 89.3% exhibited phenotypic resistance to carbapenems by disk diffusion, whereas only 39.3% harbored detectable carbapenemase genes by PCR. This discrepancy is likely due not to the low sensitivity of the PCR method but to the possibility that resistance may be mediated by other genes, or that mutations are present in gene regions corresponding to the primer binding sites in the studied isolates.

In contrast, among Escherichia coli strains isolated from broiler chickens, carbapenemase genes were detected by PCR in 64.3% of isolates, whereas only 57.1% exhibited phenotypic carbapenem resistance by microbiological testing. This discrepancy may reflect the presence of carbapenemase genes that do not confer detectable resistance under standard testing conditions—potentially due to low expression levels, lack of accompanying permeability defects (e.g., porin loss), or the presence of non-functional gene variants.

When analyzing the prevalence of genes encoding resistance to aminoglycosides (aac(6')-II and aph(3')-VI), resistance was recorded by the microbiological method in 10.7% of strains isolated from AII patients, whereas PCR detected aminoglycoside resistance genes in 21.4% of strains isolated from broilers.

4. Conclusions

Statistically significant differences were revealed in the prevalence of resistance genes responsible for β-lactamase and aminoglycoside resistance in E. coli strains isolated from AII patients and broiler chickens. A high degree of concordance was observed between genotype and resistance phenotype, confirming the effectiveness of molecular genetic screening methods for rapid and accurate monitoring. The results obtained emphasize the necessity of coordinated control of human and animal health within the framework of the “One Health” concept.

Our study covered the main and most prevalent resistance genes; however, for a more comprehensive picture, it should also be considered that additional resistance genes exist, such as AmpC, OXA, GES, qnr, rmt, and others.

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